Monitoring immunosuppression after liver transplantation : development of individualized Bayesian limited sampling monitoring

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CHAPTER 4

INDIVIDUALIZED POPULATION PHARMACO-KINETIC MODEL WITH LIMITED SAMPLING FOR CYCLOSPORINE MONITORING AFTER LIVER TRANSPLANTATION IN CLINICAL PRACTICE: C0+C2?

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Alimentary Pharmacology and Therapeutics, 2007 Nov 15;26(10): pp 1447-54

ABSTRACT

Background: We recently developed and validated limited sampling models (LSMs) for cyclosporine monitoring after orthotopic liver transplantation based on individualized population pharmacokinetic models with Bayesian modelling.

Aim: To evaluate LSM in practice, and to seek optimal balance between benefit and discomfort.

Methods: In 30 stable patients, more than 6 months after orthotopic liver transplantation, previously switched from trough- to 2 h post-dose (C2)-monitoring, we switched to 3-monthly LSM 0,1,2,3 h-monitoring. During 18 months we evaluated dose, creatinine clearance, calculated area under the curve, intra-patient pharmacokinetic variability and ability to assess systemic exposure by several previously validated LSMs.

Results: Within patients, there was variability of cyclosporine-area under the curve with the same dose (CV of 15%). Compared to C2-monitoring, there was no significant difference in dose ($P = 0.237$), creatinine clearance ($P = 0.071$) and number of rejections. Some models showed excellent correlation and precision with LSM 0,1,2,3 h comparing area under the curves (0,2 h: $r^2 = 0.88$; 0,1,3 h: $r^2 = 0.91$; 0,2,3 h: $r^2 = 0.92$, all $P < 0.001$) with no difference in advised dose.

Conclusion: The limited sampling model, with only trough- and 2-h sampling, yields excellent accuracy and assesses systemic exposure much better than C2 with less bias and greater precision. Considering the calculated intra-patient variability, more precision is redundant, so LSM 0,2 h seems the optimal way of cyclosporine-monitoring.

INTRODUCTION

Calcineurin inhibitors like cyclosporine are frequently used after solid organ transplantation such as orthotopic liver transplantation (OLT). However, these drugs are characterized by a narrow therapeutic range with risks of overdosing and underdosing. For this reason systemic exposure of this drug is routinely assessed. Several methods with varying complexity and performance exist. Until recently most clinics used trough-level monitoring (C0) to assess systemic exposure to cyclosporine, but over the last years many centres replaced this method by so-called C2-monitoring, where blood samples were taken exactly 2 h after oral administration of the drug[1-10]. This method has been shown superior in predicting the area under the curve (AUC) and toxicity. In a previous study, in stable patients more than 6 months after OLT, we demonstrated lowering of the dose in two-thirds of the patients with improved kidney function when switching from C0- to C2-monitoring[11]. However, a substantial percentage of underdosing occurred with this method, suggesting the need for even better monitoring methods.

We then developed and validated flexible limited sampling models (LSMs), based on an individualized population pharmacokinetic (PK) model, limited sampling and Bayesian estimations, which was again superior to C2.[12] All patients who were previously switched from C0- to C2- cyclosporine monitoring were now switched to 3-monthly monitoring with this LSM and followed for a period of 18 months. This strategy allowed us to investigate the feasibility of implementation of LSM into daily practice, and the potential effects of the change from C2 to LSM on such factors as dose, renal function, rejection rate and also interpatient variability. Using LSM, it was possible to determine intra-patient variability in PK of cyclosporine. With this, it was possible to determine the required precision of the method used. In addition, a new target range for cyclosporine AUC based on the 95% confidence interval for clearance could be calculated.

PATIENT AND METHODS

Thirty stable patients more than 1 year after OLT (20 men, mean age 54, range 34–66; 10 women, mean age 42, range 22–61) received the micro-emulsion formulation of cyclosporine (Neoral; Novartis Pharmaceuticals, Basel, Switzerland) twice daily as immunosuppressant. The reasons for OLT were cirrhosis due to hepatitis B-virus (four patients), alcoholic liver disease (seven patients), primary biliary cirrhosis (one patient), hepatitis C-virus (five patients), primary sclerosing cholangitis (one patient), Budd Chiari syndrome (two patients), autoimmune-hepatitis (one patient), Wilson's disease (two patients), hepatocellular carcinoma (three patients), neuroendocrine

tumour (one patient), acute fatty liver of pregnancy (one patient) and two patients with acute liver failure with unknown aetiology.

During the study, one patient sometimes had aminotransferases just above the upper limit of normal, probably as signs of reactivation of hepatitis C-virus, but this was not the case on a 3-monthly cyclosporine monitoring day. No cases of hematuria or proteinuria occurred. The mean time of exposure to cyclosporine prior to entry in this study was 46 ± 26 months (range 12–109). Six patients showed rejections between the time of OLT and time of starting this study, but all were stable again when entering the study.

After informed consent, the patients came to the clinic for check-up and blood samples were taken for cyclosporine concentration close to 0 h, 1 h, 2 h and 3 h after the morning dose of cyclosporine, while still on C2- monitoring. Whole blood cyclosporine concentrations were determined by Fluorescence Polarisation Immuno Assay (FPIA, Axsym; Abbott Diagnostics, Abbott Park, IL, USA).

Then cyclosporine dose was adjusted based on AUC calculation of LSM 0,1,2,3 h[12]. AUCs were calculated using the following formula:

*AUC = (F_po * dose * 1000) / clearance*, in which F_po is the bioavailability which is fixed at 0.5 for cyclosporine micro-emulsion, dose is the morning dose of cyclosporine and clearance is the clearance of cyclosporine calculated for the combination of time points 0 + 1 + 2 + 3 h using the PK software package MW\Pharm version 3.50 (Mediware, Groningen, The Netherlands)[13].

After every limited sampling curve, a dose advice was given using the formula:

*Advised dose = (target AUC / calculated AUC) * dose*, in which 3350 is set as value for the target AUC [middle of target-range, (2900 + 3800) / 2], which is based on the range of trough-level monitoring of 90–125 μg/L[11]. If allowed by renal function (CRCL > 50 mL/min) the dose was adjusted to the advised dose. Every 3 months thereafter LSM 0,1,2,3 h was obtained and cyclosporine dose adjusted accordingly. After dose adjustment an extra curve was obtained. From the 30 patients in total 152 LSM 0,1,2,3 h-curves (mean per patient 5 ± 2, range 1–9) were collected over the last 18 months. Four patients changed to other immunosuppressive medication during the course of this study (one because of rejection, two because of renal dysfunction, one because of gum hyperplasia).

Blood samples were also taken for kidney- and liver function. Creatinine clearance (CRCL) was calculated with Cockcroft & Gault formula. As warranted by our liver transplant protocol, a liver biopsy was obtained when rejection was suspected. Moderate-to-severe rejection was treated with additional immune suppression, while in mild rejection the dose of maintenance immune suppression was optimized.

Intra-patient variability in clearance (CV%) was investigated calculating the mean and standard deviation of the clearance of all curves for all patients using the formula: *variation coefficient = (standard deviation / mean clearance) * 100%*.
In order to create a new target-range for the AUC, a 95% confidence interval for clearance was calculated using the formula:
*AUC = (0.5*dose*1000) / (clearance ± 2s.d.)*

Statistics

Statistical analysis on patient data was performed using SPSS 11.0.1 for Windows (SPSS Inc., Chicago, IL, USA). Results are expressed as mean ± s.d. and as median and range. Potential differences were explored with Paired Samples *t*-test and relationships were investigated using Pearson correlation test and Pearson chi-squared test. *P*-values below 0.05 were considered to be statistically significant. AUCs were calculated using previously developed and validated LSMs[12].
The calculated AUCs, based on a single-point and combinations of blood sampling time points, were compared with the AUC based on time points 0 + 1 + 2 + 3 h by Pearson correlation test. Predictive performance of this method was also investigated by calculating the prediction precision and bias according to procedures developed by Sheiner and Beal[14]. Prediction bias was calculated as the mean prediction error (MPE), this is the mean of differences between the AUCs of the different models and the AUC based on time points 0 + 1+2 + 3 h. Prediction precision was calculated as the mean absolute prediction error (MAPE), this is the mean of the absolute differences between the AUCs of the different models and the AUC based on time points 0 + 1 + 2 + 3 h. Smaller values for MPE and MAPE indicate less bias and greater precision (acceptable ranges ± 10%).

RESULTS

Time to reach peak concentration

While monitoring cyclosporine concentration in blood there was a difference between patients and also within patients concerning the time to reach peak concentration of cyclosporine. From all the 152 curves we obtained, there were 69 curves (45%) with a peak on C1, 71 curves (47%) on C2 and 12 curves (8%) on C3 (Table 1). Table 2 shows a few examples of the results per patient demonstrating considerable intra-patient variability.
Based on these results we may conclude that monitoring only on C2 is not reflecting the AUC well enough because of intra-patient variation in time after dosing to reach peak concentrations.

Table 1. Time of the peak cyclosporine blood concentration of all 152 limited sampling model 0,1,2,3 h-curves

Sampling point	Number of peaks	(%)
C0	0	0
C1	69	45
C2	71	47
C3	12	8
total	152	100

Table 2. Number of limited sampling model 0,1,2,3 h curves with peak cyclosporine level at C0, C1, C2 or C3 per patient for three patients.

Patient number	Peak C0	Peak C1	Peak C2	Peak C3	Number of curves
3	0	3	2	0	5
9	0	1	5	3	9
21	0	3	3	2	8
Total	0	7	10	5	22

Variation of clearance

Calculating the variation coefficient (CV%) for every patient using the mean clearance and standard deviation of all curves this CV% was 15%. Mean dose of all patients was 109 mg twice daily, so natural variation in one patient is 109*0.15 = 16 mg.
We calculated a 95% confidence interval for the clearance in order to create a new target range, which is based on natural variation. This new target range is 2380–4390 h*µg/L, much wider than the target range we use in our clinic (2900–3800 h*µg/L). Even when we use 1s.d. instead of 2s.d. the target range would be 2680–3620 h*µg/L.

Difference in dose, kidney function and rejection

Before switching from C2-monitoring to LSM 0,1,2,3 h mean cyclosporine dose, while on C2-monitoring, was 207 ± 9 mg daily (range 150–350 mg). After switching, mean daily dose was 218 ± 10 mg (range 100–300 mg). Mean change in dose was 11 ± 9 mg (P = 0.237, median 0.0, range −100 to +100), so there was no significant change of average cyclosporine dose after switching from C2-monitoring to LSM 0,1,2,3 h.
Looking at the individual patient, only two patients once had a daily-dose change of 100 mg, one −100 mg and one +100 mg. The other patients had daily-dose changes of 50 mg or less.
Mean CRCL on C2-monitoring was 77.0 ± 4.5 mL/min (range 40.4–132 mL/min). While using LSM 0,1,2,3 h mean CRCL was 73.0 ± 4.8 mL/min (range 26.6–128.8 mL/min). The difference in CRCL between C2-monitoring and LSM 0,1,2,3 h was

−4.0 ± 2.1 mL/min (P = 0.071) so on average there was no significant change of the kidney function. Looking at the individual patient level, there was a wide variability in CRCL change (range: −30.1 to +17.7, median: −5.4). Even when dividing all patients into three groups (tertiles) based on CRCL in each group there was a comparable variability of CRCL (data not shown).
While using LSM 0,1,2,3 h for 18 months, there were two moderate-to-severe rejections vs. two moderate-to-severe rejections during the previous 18 months on C2-monitoring.

Correlation of other LSMs with LSM 0,1,2,3 h

For the LSM 0,1,2,3 h model and for the models with time points 0 h, 1 h, 2 h and 3 h and the combinations of time points 0,1 h, 0,2 h, 0,3 h, 1,2 h, 1,3 h, 2,3 h, 0,1,2 h, 0,1,3 h, 0,2,3 h and 1,2,3 h we calculated for all 152 curves the AUC and the correlation with LSM 0,1,2,3 h (Table 3). Correlation of AUC calculated with LSM 0,1,2,3 h for other multiple-point models was much better than LSM 0 h and LSM 2 h. Two 2-point-models showed good correlation with our 0,1,2,3 h model:
LSM 0,2 h (r^2 = 0.88) and LSM 0,3 h (r^2 = 0.87) with acceptable bias and precision.
Three 3-point-models also showed good correlation with acceptable bias and precision:
LSM 0,1,2 h (r^2 = 0.84), LSM 0,1,3 h (r^2 = 0.91) and LSM 0,2,3 h (r^2 = 0.92) (Figure 1). Of special interest is the important contribution of the trough-level (C0), which seems to be indispensable for adequate monitoring of cyclosporine in combination with at least one other sampling point.

Table 3. Correlation of other individualized pharmacokinetic (PK) models [limited sampling models (LSMs)] with LSM 0,1,2,3 h

Individualized PK-model (LSM) with sampling on time (h):	r^2	MPE (%)	MAPE (%)
0	0.67	7	16
1	0.12	8	24
2	0.50	−6	13
3	0.66	3	11
0,1	0.69	10	18
0,2	0.88	−2	6
0,3	0.87	5	9
1,2	0.42	−9	16
1,3	0.72	1	9
2,3	0.68	−2	9
0,1,2	0.84	−2	6
0,1,3	0.91	4	6
0,2,3	0.92	2	5
1,2,3	0.67	−4	9
0,1,2,3	1.00	0	0

MPE, mean prediction error; MAPE, mean absolute prediction error.

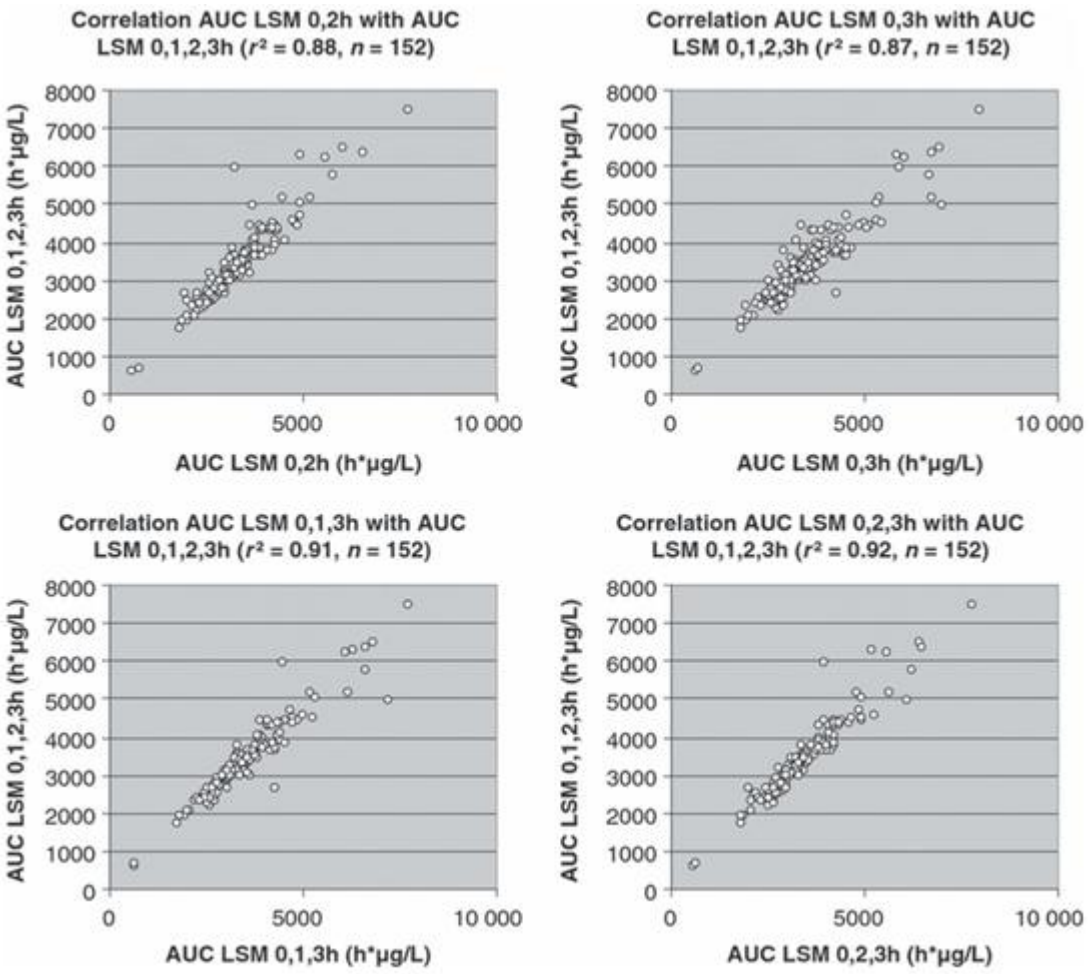

Figure 1. Correlation of area under the curves (AUCs) limited sampling model (LSM) 0,2 h, LSM 0,3 h, LSM 0,1,3 h and LSM 0,2,3 h with AUC LSM 0,1,2,3 h.

We then calculated the correlation of these five two-point and three-point LSMs with LSM 0,1,2,3 h per patient. Looking only at the 20 patients with at least five 0,1,2,3 h curves, in 19/20 patients we see a high and significant correlation of AUCs calculated with LSM 0,1,2,3 h and those AUCs calculated with the models LSM 0,2 h, LSM 0,1,2 h, LSM 0,1,3 h and LSM 0,2,3 h. For the LSM 0,3 h in 16/20 patients there was a good and significant correlation with LSM 0,1,2,3 h (Table 4).

Table 4. Correlation of area under the curve (AUC) calculated with different limited sampling models (LSMs) with AUC calculated with LSM 0,1,2,3 h per patient with five or more curves

LSM (h)	n with $P < 0.05$	r^2 (range)
0,2	19/20	0.81–0.99
0,3	16/20	0.80–1.00
0,1,2	19/20	0.84–1.00
0,1,3	19/20	0.67–1.00
0,2,3	19/20	0.82–1.00

These correlations suggest that these other LSMs with less time points show results comparable to LSM 0,1,2,3 h and that particularly LSM 0,2 h is an accurate, reliable and very practical model with acceptable bias and precision for monitoring cyclosporine, as we saw earlier when developing and validating our LSM 0,1,2,3 h and these other LSMs[12] .

DISCUSSION

This study, in the first place, shows that it is feasible to implement cyclosporine monitoring based on limited sampling and an individualized population PK model in a liver transplant out-patient clinic.
Second, we show that cyclosporine dose, renal function and rejection rate did not change significantly after our switch from C2-based monitoring to LSM 0,1,2,3 h. Third, we show that often we decided not to increase the cyclosporine dose because of renal dysfunction while we were advised to do so because the calculated AUC was below the target range, while usually no rejection followed. This means that apparently the lower limit of the target range was too high. Fourth, a significant intra-patient variation appeared to occur with the same cyclosporine dose. Fifth, several two- and three-point – previously validated- LSMs correlated very well with the four-point LSM 0,1,2,3 h. All include the trough level, which seems indispensable to get an accurate AUC prediction, as we previously showed. Sixth, the LSM 0,2 h seems optimal in terms of accuracy, ease-of-use and intra-patient variability.
Because of the narrow therapeutic range of cyclosporine, assessing the systemic exposure to this drug is mandatory. Ideally, a full AUC is measured on a regular basis. As this is not practical and C0 is a rough estimation of the AUC, for many years monitoring based on trough levels was used. Then many centers switched to monitoring based on C2, after it was shown that C2 correlates better with AUC as Citerrio describes in an article about the evolution of the therapeutic drug monitoring of cyclosporine[15]. This had the disadvantage of a fixed time point after dosing, which is difficult for some patients. Moreover, C2 still does not reflect very well the AUC and according to the review study of Marin *et al.* the best way to individualize therapy is still controversial. Recommendations are made for clinical research that could be done to provide more definitive evidence for the use of C2 or other limited sampling strategies[16]. After C0-monitoring and the more precise C2-monitoring we showed that our LSM 0,1,2,3 h-method more accurately estimates systemic exposure to cyclosporine in OLT patients, based on limited sampling, individualized population PK models and Bayesian estimations with an easy-to-use computer model[12]. LSMs have the advantage

that sampling times are not rigid in contrast to most limited sampling strategies described in a review article of David and Johnston[17]. Switching from C0- via C2-monitoring and subsequently to LSM 0,1,2,3 allowed us to compare the biochemical and clinical effects of these three methods.

There appeared to be considerable intra-patient variability of time to reach the peak-concentration of cyclosporine. This led to the same number of dose adjustments as with C2-monitoring in the 18 months before the C2 to LSM 0,1,2,3 h switch. The intra-patient PK variability may partially be due to interaction with food or other medication. The variation in peak-time is partially responsible for the large intra-patient variation in C2 levels over time in some of the patients. With an LSM with more sampling time points, all important information required for calculating an AUC is obtained and the chance of 'missing' this variability is less, which leads to more accurate AUC estimations.

After more than one-and-a-half-year of using our model for cyclosporine monitoring in the out-patient clinic, 152 LSM 0,1,2,3 h curves from 30 patients were derived. Although this is not a randomized controlled trial these stable patients were their own controls. According to the dose, renal function and rejection on average there was no difference using C2-monitoring or the individualized PK model. However, the target range was based on AUCs while on C0-monitoring. In an earlier study, while on C2-monitoring, we saw two rejections in 13 cases where the AUC dropped below the AUC target range. Apparently, an AUC below 2900 h*µg/L is tolerated in many patients. This was similar for LSM 0,1,2,3 h monitoring: for some patients the dose was not increased as advised after LSM 0,1,2,3 because renal insufficiency did not allow us to do so, but although these patients were at risk of underdosing, usually no signs of rejection occurred.

Although there was no significant change in CRCL between C2-monitoring and LSM 0,1,2,3 h, there seemed to be a trend toward lower CRCL with LSM vs. C2-monitoring (P = 0.071). More data is needed to confirm the usefulness of tailoring cyclosporine dosing by LSM to minimize toxicity.

The current data allow us to investigate the true natural variability in PK of cyclosporine in stable OLT patients. The mean intra-patient variability of the apparent oral clearance of cyclosporine in these stable liver transplantation patients was 15%. This means that a dose adjustment of 16 mg or less (15% of mean dose of 109 mg) is not rational, because this difference is a natural variation, which cannot be avoided. In fact, the lowest possible dose adjustment (25 mg) in practice is relatively close to this natural variation of 16 mg. In case the mean dose of 109 mg and a 95% confidence interval (mean ± 2*s.d.) would be used, a target range of 2380–4390 h*µg/L would be rational. In other words, any AUC value within this range can be explained by natural variability in PK of cyclosporine and may therefore not require a dose adjustment.

In our hospital, a target range of 2900–3800 h*µg/L was used for stable OLT patients, which is narrower, and closer to a mean ± 1*s.d. value of the AUC in this population, which is 2680–3620 h*µg/L. However, to be on the safe side, we until now remain adhering to this narrow range, although we realize that this may be too strict. Based on the current data, a lower range for the AUC than currently used with a target AUC of 2830 h*µg/L (2380–3280 h*µg/L) may be reasonable.

Our data suggest that, considering the natural variability in PK of cyclosporine in stable OLT patients, our method with LSM 0,1,2,3 h may be too accurate in terms of estimating systemic exposure to cyclosporine.

When investigating the correlation between LSMs with only two or three sampling points and the LSM 0,1,2,3 h we see that overall five models showed good correlation when considering both the AUCs and the mean advised dose. These five LSMs were 0,2 h; 0,3 h; 0,1,2 h; 0,1,3 h and 0,2,3 h. Accuracy and bias were acceptable. The trough level is included into all of these models, which illustrates the pivotal role of this sample for assessing systemic exposure to cyclosporine. We are aware of the fact that these five models are abbreviated curves from the already abbreviated 0,1,2,3 h curve, but recently we already noticed a very good correlation of these models with the gold standard $AUC_{0-12\ h}$ (for LSM 0,2 h this was: $r^2 = 0.94$, MPE = −9, MAPE = 9) with less bias and greater precision than e.g. C2 single-point monitoring ($r^2 = 0.78$, MPE = −10, MAPE = 12) or Ctrough[12].

In spite of the fact that LSM 0,1,2 h includes both the common 1- and 2-h peak-level time points, the correlation of this model with LSM 0,1,2,3 h in the patients with five or more curves is not different from LSM 0,2 h (r^2 = 0.84–1.00 vs. 0.81–0.99).

Comparing LSM 0,1,2 h with LSM 0,2 h, the 0,2 h-model has the benefit that it is easier to apply in practice, it is more friendly for the patient and the medical staff, and there is a cost-benefit. Therefore this model seems an optimal balance between benefit and discomfort for the patient. A large randomized controlled trial between C2 and LSM 0,2 h with a target AUC of 2830 h*µg/L (range 2380–3280 h*µg/L) would be of interest.

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